Exogenous Lysyl Oxidase-Like 2 and Perfusion Culture Induce Collagen Crosslink Formation in Osteogenic Grafts.

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Public Summary:
Tissue engineering represents a promising approach to generate functional, implantable tissues to address tissue lost due to disease, trauma, or aging. Current approaches for generating tissue engineered bone often seed engineered implants with bone-forming cells for subsequent implantation, depending upon native processes in the bone defect to guide the maturation and remodeling of implanted tissue. As an alternative, the preconditioning of cellularized bone grafts in bioreactors prior to implantation provides an opportunity to generate implants with improved bone-forming potential and mechanical properties. Numerous factors contribute to the success of this approach including the identity and density of seeded cells, composition of media to direct cell fate, and flow rate and profile of media. In addition, the supplementation of culture media with collagen crosslink-inducing biomolecules may provide a novel strategy for generating more mature bone grafts for subsequent implantation. Lysyl oxidase (LOX)-mediated collagen crosslinking can regulate osteoblastic phenotype and enhance mechanical properties of tissues, both areas of interest in bone tissue engineering. As a first step toward this goal, we investigated the effect of lysyl oxidase-like 2 (LOXL2) on osteogenic differentiation of mesenchymal stem cells (MSCs) cultured in perfusion bioreactors, enzymatic collagen crosslink formation in the extracellular matrix (ECM), and mechanical properties of engineered bone grafts. MSC-seeded composite scaffolds were exposed to exogenous LOXL2 and maintained under perfusion culture for up to 28 days. Constructs treated with LOXL2 possessed greater DNA content, an indicator of cell number, and osteogenic potential measured by a two-fold increase in bone sialoprotein gene expression. Collagen expression of LOXL2-treated scaffolds was lower than untreated controls. Functional outputs such as calcium deposition, osteocalcin expression, and compressive modulus were unaffected by LOXL2 supplementation. Excitingly, LOXL2-treated constructs contained 1.8- and 1.4-times more pyridinoline (PYD) collagen crosslinks per mole of collagen and per wet weight, respectively, than untreated constructs. Despite these increases, compressive moduli of LOXL2-treated constructs were similar to untreated constructs over the 28-day culture duration. These data suggest that exogenous LOXL2 supplementation was ineffective in accelerating osteogenic differentiation over constructs maintained in perfusion and in osteogenic media, yet this approach successfully increased the quantity of collagen crosslinks. This is the first report of LOXL2 application to engineered, three-dimensional bony constructs. The results suggest a potentially new strategy for engineering osteogenic grafts with a mature ECM by modulating crosslink formation.

Scientific Abstract:
Lysyl oxidase (LOX)-mediated collagen crosslinking can regulate osteoblastic phenotype and enhance mechanical properties of tissues, both areas of interest in bone tissue engineering. The objective of this study is to investigate the effect of lysyl oxidase-like 2 (LOXL2) on osteogenic differentiation of mesenchymal stem cells (MSCs) cultured in perfusion bioreactors, enzymatic collagen crosslink formation in the extracellular matrix (ECM), and mechanical properties of engineered bone grafts. Exogenous LOXL2 to MSCs seeded in composite scaffolds under perfusion culture for up to 28 days is administered. Constructs treated with LOXL2 appear brown in color and possess greater DNA content and osteogenic potential measured by a twofold increase in bone sialoprotein gene expression. Collagen expression of LOXL2-treated scaffolds is lower than untreated controls. Functional outputs such as calcium deposition, osteocalcin expression, and compressive modulus are unaffected by LOXL2 supplementation. Excitingly, LOXL2-treated constructs contain 1.8- and 1.4-times more pyridinoline (PYD) crosslinks per mole of collagen and per wet weight, respectively, than untreated constructs. Despite these increases, compressive moduli of LOXL2-treated constructs are similar to untreated constructs over the 28-day culture duration. This is the first report of LOXL2 application to engineered, three-dimensional bony constructs. The results suggest a potentially new strategy for engineering osteogenic grafts with a mature ECM by modulating crosslink formation.

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